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(54) Title: NUCLEIC ACIDS AND PROTEINS FROM STREPTOCOCCUS GROUPS A & B

(57) Abstract: The invention provides proteins from group B streptococcus (*Streptococcus agalactiae*) and group A streptococcus (*Streptococcus pyogenes*), including amino acid sequences and the corresponding nucleotide sequences. Data are given to show that the proteins are useful antigens for vaccines, immunogenic compositions, and/or diagnostics. The proteins are also targets for antibiotics.

NUCLEIC ACIDS AND PROTEINS FROM STREPTOCOCCUS GROUPS A & B

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention relates to nucleic acid and proteins from the bacteria *Streptococcus agalactiae* (GBS) and *Streptococcus pyogenes* (GAS).

BACKGROUND ART

Once thought to infect only cows, the Gram-positive bacterium *Streptococcus agalactiae* (or "group B streptococcus", abbreviated to "GBS") is now known to cause serious disease, bacteremia and meningitis, in immunocompromised individuals and in neonates. There are two types of neonatal infection. The first (early onset, usually within 5 days of birth) is manifested by bacteremia and pneumonia. It is contracted vertically as a baby passes through the birth canal. GBS colonises the vagina of about 25% of young women, and approximately 1% of infants born via a vaginal birth to colonised mothers will become infected. Mortality is between 50-70%. The second is a meningitis that occurs 10 to 60 days after birth. If pregnant women are vaccinated with type III capsule so that the infants are passively immunised, the incidence of the late onset meningitis is reduced but is not entirely eliminated.

The "B" in "GBS" refers to the Lancefield classification, which is based on the antigenicity of a carbohydrate which is soluble in dilute acid and called the C carbohydrate. Lancefield identified 13 types of C carbohydrate, designated A to O, that could be serologically differentiated. The organisms that most commonly infect humans are found in groups A, B, D, and G. Within group B, strains can be divided into 8 serotypes (Ia, Ib, Ia/c, II, III, IV, V, and VI) based on the structure of their polysaccharide capsule.

Group A streptococcus ("GAS", *S.pyogenes*) is a frequent human pathogen, estimated to be present in between 5-15% of normal individuals without signs of disease. When host defences are compromised, or when the organism is able to exert its virulence, or when it is introduced to vulnerable tissues or hosts, however, an acute infection occurs. Diseases include puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotising fasciitis, myositis and streptococcal toxic shock syndrome.

S.pyogenes is typically treated using antibiotics. Although *S.agalactiae* is inhibited by antibiotics, however, it is not killed by penicillin as easily as GAS. Prophylactic vaccination is thus preferable.

Current GBS vaccines are based on polysaccharide antigens, although these suffer from poor immunogenicity. Anti-idiotypic approaches have also been used (*e.g.* WO99/54457). There remains a need, however, for effective adult vaccines against *S.agalactiae* infection. There also remains a need for vaccines against *S.pyogenes* infection.

It is an object of the invention to provide proteins which can be used in the development of such vaccines. The proteins may also be useful for diagnostic purposes, and as targets for antibiotics.

DISCLOSURE OF THE INVENTION

The invention provides proteins comprising the *S.agalactiae* amino acid sequences disclosed in the examples, and proteins comprising the *S.pyogenes* amino acid sequences disclosed in the examples. These amino acid sequences are the even SEQ IDs between 1 and 10960.

It also provides proteins comprising amino acid sequences having sequence identity to the *S.agalactiae* amino acid sequences disclosed in the examples, and proteins comprising amino acid sequences having sequence identity to the *S.pyogenes* amino acid sequences disclosed in the examples. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more). These proteins include homologs, orthologs, allelic variants and functional mutants. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*.

Preferred proteins of the invention are GBS1 to GBS689 (see Table IV).

The invention further provides proteins comprising fragments of the *S.agalactiae* amino acid sequences disclosed in the examples, and proteins comprising fragments of the *S.pyogenes* amino acid sequences disclosed in the examples. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more). Preferably the fragments comprise one or more epitopes from the sequence. Other preferred fragments are (a) the N-terminal signal peptides of the proteins disclosed in the examples, (b) the proteins disclosed in the examples, but without their N-terminal signal peptides, (c) fragments common to the related GAS and GBS proteins disclosed in the examples, and (d) the proteins disclosed in the examples, but without their N-terminal amino acid residue.

The proteins of the invention can, of course, be prepared by various means (*e.g.* recombinant expression, purification from GAS or GBS, chemical synthesis *etc.*) and in various forms (*e.g.* native, fusions, glycosylated, non-glycosylated *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other streptococcal or host cell proteins) or substantially isolated form. Proteins of the invention are preferably streptococcal proteins.

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means (*e.g.* by recombinant expression). To increase compatibility with the human immune system, the antibodies may be chimeric or humanised (*e.g.* Breedveld (2000) *Lancet* 355(9205):735-740; Gorman & Clark (1990) *Semin. Immunol.* 2:457-466), or fully human antibodies may be used. The antibodies may include a detectable label (*e.g.* for diagnostic assays).

According to a further aspect, the invention provides nucleic acid comprising the *S.agalactiae* nucleotide sequences disclosed in the examples, and nucleic acid comprising the *S.pyogenes* nucleotide sequences disclosed in the examples. These nucleic acid sequences are the odd SEQ IDs between 1 and 10966.

In addition, the invention provides nucleic acid comprising nucleotide sequences having sequence identity to the *S.agalactiae* nucleotide sequences disclosed in the examples, and nucleic acid comprising nucleotide sequences having sequence identity to the *S.pyogenes* nucleotide sequences disclosed in the examples. Identity between sequences is preferably determined by the Smith-Waterman homology search algorithm as described above.

Furthermore, the invention provides nucleic acid which can hybridise to the *S.agalactiae* nucleic acid disclosed in the examples, and nucleic acid which can hybridise to the *S.pyogenes* nucleic acid disclosed in the examples preferably under 'high stringency' conditions (*e.g.* 65°C in 0.1xSSC, 0.5% SDS solution).

Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least *n* consecutive nucleotides from the *S.agalactiae* or *S.pyogenes* sequences and, depending on the particular sequence, *n* is 10 or more (*e.g.* 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). The fragments may comprise sequences which are common to the related GAS and GBS sequences disclosed in the examples.

According to a further aspect, the invention provides nucleic acid encoding the proteins and protein fragments of the invention.

The invention also provides: nucleic acid comprising nucleotide sequence SEQ ID 10967; nucleic acid comprising nucleotide sequences having sequence identity to SEQ ID 10967; nucleic acid which can hybridise to SEQ ID 10967 (preferably under 'high stringency' conditions); nucleic acid comprising a fragment of at least *n* consecutive nucleotides from SEQ ID 10967, wherein *n* is 10 or more *e.g.* 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1500, 2000, 3000, 4000, 5000, 10000, 100000, 1000000 or more'

Nucleic acids of the invention can be used in hybridisation reactions (*e.g.* Northern or Southern blots, or in nucleic acid microarrays or 'gene chips') and amplification reactions (*e.g.* PCR, SDA, SSSR, LCR, TMA, NASBA *etc.*) and other nucleic acid techniques.

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing, or for use as primers).

Nucleic acid according to the invention can, of course, be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, primers, probes, labelled *etc.*). The nucleic acid is preferably in substantially isolated form.

Nucleic acid according to the invention may be labelled *e.g.* with a radioactive or fluorescent label. This is particularly useful where the nucleic acid is to be used in nucleic acid detection techniques *e.g.* where the nucleic acid is a primer or as a probe for use in techniques such as PCR, LCR, TMA, NASBA *etc.*

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*e.g.* cloning or expression vectors) and host cells transformed with such vectors.

According to a further aspect, the invention provides compositions comprising protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as immunogenic compositions, for instance, or as diagnostic reagents, or as vaccines.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (*e.g.* as immunogenic compositions or as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of: (i) a medicament for treating or preventing disease and/or infection caused by streptococcus; (ii) a diagnostic reagent for detecting the presence of streptococcus or of antibodies raised against streptococcus; and/or (iii) a reagent which can raise antibodies against streptococcus. Said streptococcus may be any species, group or strain, but is preferably *S.agalactiae*, especially serotype III or V, or *S.pyogenes*. Said disease may be bacteremia, meningitis, puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotising fasciitis, myositis or toxic shock syndrome.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody of the invention. The patient may either be at risk from the disease themselves or may be a pregnant woman ('maternal immunisation' *e.g.* Glezen & Alpers (1999) *Clin. Infect. Dis.* 28:219-224).

Administration of protein antigens is a preferred method of treatment for inducing immunity.

Administration of antibodies of the invention is another preferred method of treatment. This method of passive immunisation is particularly useful for newborn children or for pregnant women. This method will typically use monoclonal antibodies, which will be humanised or fully human.

The invention also provides a kit comprising primers (*e.g.* PCR primers) for amplifying a template sequence contained within a *Streptococcus* (*e.g. S.pyogenes* or *S.agalactiae*) nucleic acid sequence, the kit comprising a first primer and a second primer, wherein the first primer is substantially complementary to said template sequence and the second primer is substantially complementary to a complement of said template sequence, wherein the parts of said primers which have substantial complementarity define the termini of the template sequence to be amplified. The first primer and/or the second primer may include a detectable label (*e.g.* a fluorescent label).

The invention also provides a kit comprising first and second single-stranded oligonucleotides which allow amplification of a *Streptococcus* template nucleic acid sequence contained in a single- or double-stranded nucleic acid (or mixture thereof), wherein: (a) the first oligonucleotide comprises a primer sequence which is substantially complementary to said template nucleic acid sequence; (b) the second oligonucleotide comprises a primer sequence which is substantially complementary to the complement of said template nucleic acid sequence; (c) the first oligonucleotide and/or the second oligonucleotide comprise(s) sequence which is not compementary to said template nucleic acid; and (d) said primer sequences define the termini of the template sequence to be amplified. The non-complementary sequence(s) of feature (c) are preferably upstream of (*i.e.* 5' to) the primer sequences. One or both of these (c) sequences may comprise a restriction site (*e.g.* EP-B-0509612) or a promoter sequence (*e.g.* EP-B-0505012). The first oligonucleotide and/or the second oligonucleotide may include a detectable label (*e.g.* a fluorescent label).

The template sequence may be any part of a genome sequence (*e.g.* SEQ ID 10967). For example, it could be a rRNA gene (*e.g.* Turenne *et al.* (2000) *J. Clin. Microbiol.* 38:513-520; SEQ IDs 12018-12024 herein) or a protein-coding gene. The template sequence is preferably specific to GBS.

The invention also provides a computer-readable medium (*e.g.* a floppy disk, a hard disk, a CD-ROM, a DVD *etc.*) and/or a computer database containing one or more of the sequences in the sequence listing. The medium preferably contains SEQ ID 10967.

The invention also provides a hybrid protein represented by the formula NH_2-A-[-X-L-]$_n$-B-COOH, wherein X is a protein of the invention, L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and n is an integer greater than 1. The value of n is between 2 and x, and the value of x is typically 3, 4, 5, 6, 7, 8, 9 or 10. Preferably n is 2, 3 or 4; it is more preferably 2 or 3; most preferably, n = 2. For each n instances, -X- may be the same or different. For each n instances of [-X-L-], linker amino acid sequence -L- may be present or absent. For instance, when n=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. -A- and -B- are optional sequences which will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 10 or more). Other suitable N-terminal and C-terminal amino acid sequences will be appare-

skilled in the art. In some embodiments, each X will be a GBS sequence; in others, mixtures of GAS and GBS will be used.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell of to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridising conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting *Streptococcus* in a biological sample (*e.g.* blood) is also provided, comprising the step of contacting nucleic acid according to the invention with the biological sample under hybridising conditions. The process may involve nucleic acid amplification (*e.g.* PCR, SDA, SSSR, LCR, TMA, NASBA *etc.*) or hybridisation (*e.g.* microarrays, blots, hybridisation with a probe in solution *etc.*). PCR detection of *Streptococcus* in clinical samples, in particular *S.pyogenes*, has been reported [see *e.g.* Louie *et al.* (2000) *CMAJ* 163:301-309; Louie *et al.* (1998) *J. Clin. Microbiol.* 36:1769-1771]. Clinical assays based on nucleic acid are described in general in Tang *et al.* (1997) *Clin. Chem.* 43:2021-2038.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody of the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

A process for identifying an amino acid sequence is provided, comprising the step of searching for putative open reading frames or protein-coding regions within a genome sequence of *S.agalactiae*. This will typically involve *in silico* searching the sequence for an initiation codon and for an in-frame termination codon in the downstream sequence. The region between these initiation and termination codons is a putative protein-coding sequence. Typically, all six possible reading frames will be searched. Suitable software for such analysis includes ORFFINDER (NCBI), GENEMARK [Borodovsky & McIninch (1993) *Computers Chem.* 17:122-133), GLIMMER [Salzberg *et al.* (1998) *Nucleic Acids Res.* 26:544-548; Salzberg *et al.* (1999) *Genomics* 59:24-31; Delcher *et al.* (1999) *Nucleic Acids Res.* 27:4636-4641], or other software which uses Markov models [*e.g.* Shmatkov *et al.* (1999) *Bioinformatics* 15:874-876]. The invention also provides a protein comprising the identified amino acid sequence. These proteins can then expressed using conventional techniques.

The invention also provides a process for determining whether a test compound binds to a protein of the invention. If a test compound binds to a protein of the invention and this binding inhibits the life cycle of the GBS bacterium, then the test compound can be used as an antibiotic or as a lead compound for the

design of antibiotics. The process will typically comprise the steps of contacting a test compound with a protein of the invention, and determining whether the test compound binds to said protein. Preferred proteins of the invention for use in these processes are enzymes (*e.g.* tRNA synthetases), membrane transporters and ribosomal proteins. Suitable test compounds include proteins, polypeptides, carbohydrates, lipids, nucleic acids (*e.g.* DNA, RNA, and modified forms thereof), as well as small organic compounds (*e.g.* MW between 200 and 2000 Da). The test compounds may be provided individually, but will typically be part of a library (*e.g.* a combinatorial library). Methods for detecting a binding interaction include NMR, filter-binding assays, gel-retardation assays, displacement assays, surface plasmon resonance, reverse two-hybrid *etc.* A compound which binds to a protein of the invention can be tested for antibiotic activity by contacting the compound with GBS bacteria and then monitoring for inhibition of growth. The invention also provides a compound identified using these methods.

The invention also provides a composition comprising a protein or the invention and one or more of the following antigens:

- a protein antigen from *Helicobacter pylori* such as VacA, CagA, NAP, HopX, HopY [*e.g.* WO98/04702] and/or urease.
- a protein antigen from *N.meningitidis* serogroup B, such as those in WO99/24578, WO99/36544, WO99/57280, WO00/22430, Tettelin *et al.* (2000) *Science* 287:1809-1815, Pizza *et al.* (2000) *Science* 287:1816-1820 and WO96/29412, with protein '287' and derivatives being particularly preferred.
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in WO01/52885; Bjune *et al.* (1991) *Lancet* 338(8775):1093-1096; Fukasawa *et al.* (1999) *Vaccine* 17:2951-2958; Rosenqvist *et al.* (1998) *Dev. Biol. Stand.* 92:323-333 *etc.*
- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in Costantino *et al.* (1992) *Vaccine* 10:691-698from serogroup C [see also Costantino *et al.* (1999) *Vaccine* 17:1251-1263].
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* Watson (2000) *Pediatr Infect Dis J* 19:331-332; Rubin (2000) *Pediatr Clin North Am* 47:269-285, v; Jedrzejas (2001) *Microbiol Mol Biol Rev* 65:187-207].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* Bell (2000) *Pediatr Infect Dis J* 19:1187-1188; Iwarson (1995) *APMIS* 103:321-326].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* Gerlich *et al.* (1990) *Vaccine* 8 Suppl:S63-68 & 79-80].
- an antigen from hepatitis C virus [*e.g.* Hsu *et al.* (1999) *Clin Liver Dis* 3:901-915].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or

agglutinogens 2 and 3 [*e.g.* Gustafsson *et al.* (1996) *N. Engl. J. Med.* 334:349-355; Rappuoli *et al.* (1991) *TIBTECH* 9:232-238].

- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of *Vaccines* (1988) eds. Plotkin & Mortimer. ISBN 0-7216-1946-0] *e.g.* the CRM_{197} mutant [*e.g.* Del Guidice *et al.* (1998) *Molecular Aspects of Medicine* 19:1-70].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of Plotkin & Mortimer].
- a saccharide antigen from *Haemophilus influenzae* B.
- an antigen from *N.gonorrhoeae* [*e.g.* WO99/24578, WO99/36544, WO99/57280].
- an antigen from *Chlamydia pneumoniae* [*e.g.* PCT/IB01/01445; Kalman *et al.* (1999) *Nature Genetics* 21:385-389; Read *et al.* (2000) *Nucleic Acids Res* 28:1397-406; Shirai *et al.* (2000) *J. Infect. Dis.* 181(Suppl 3):S524-S527; WO99/27105; WO00/27994; WO00/37494].
- an antigen from *Chlamydia trachomatis* [*e.g.* WO99/28475].
- an antigen from *Porphyromonas gingivalis* [*e.g.* Ross *et al.* (2001) *Vaccine* 19:4135-4142].
- polio antigen(s) [*e.g.* Sutter *et al.* (2000) *Pediatr Clin North Am* 47:287-308; Zimmerman & Spann (1999) *Am Fam Physician* 59:113-118, 125-126] such as IPV or OPV.
- rabies antigen(s) [*e.g.* Dreesen (1997) *Vaccine* 15 Suppl:S2-6] such as lyophilised inactivated virus [*e.g. MMWR Morb Mortal Wkly Rep* 1998 Jan 16;47(1):12, 19; RabAvert™].
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of Plotkin & Mortimer].
- influenza antigen(s) [*e.g.* chapter 19 of Plotkin & Mortimer], such as the haemagglutinin and/or neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [*e.g.* McMichael (2000) *Vaccine* 19 Suppl 1:S101-107].
- an antigen from *Staphylococcus aureus* [*e.g.* Kuroda *et al.* (2001) *Lancet* 357(9264):1225-1240; see also pages 1218-1219].

Where a saccharide or carbohydrate antigen is included, it is preferably conjugated to a carrier protein in order to enhance immunogenicity [*e.g.* Ramsay *et al.* (2001) *Lancet* 357(9251):195-196; Lindberg (1999) *Vaccine* 17 Suppl 2:S28-36; *Conjugate Vaccines* (eds. Cruse *et al.*) ISBN 3805549326, particularly vol. 10:48-114 *etc.*]. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [*e.g.* EP-0372501], synthetic peptides [*e.g.* EP-0378881, EP-0427347], heat shock proteins [*e.g.* WO93/17712], pertussis proteins [*e.g.* WO98/58668; EP-0471177], protein D from *H.influenzae* [*e.g.* WO00/56360], toxin A or B from *C.difficile* [*e.g.* WO00/61761], *etc.* Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens are preferably adsorbed to an aluminium salt.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

The invention also provides compositions comprising two or more proteins of the present invention. The two or more proteins may comprise GBS sequences or may comprise GAS and GBS sequences.

A summary of standard techniques and procedures which may be employed to perform the invention (*e.g.* to utilise the disclosed sequences for vaccination or diagnostic purposes) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

General

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature *eg.* Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and II* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a streptococcus sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature

A fragment of SEQ ID NO. 1 - at least 15 bases long
A complement of SEQ ID NO. 1.

ALIGNM...

RESULT 1
ABN66737
ID ABN66737 standard; DNA; 1332 BP.
XX
AC ABN66737;
XX
DT 01-JUL-2002 (first entry)
XX
DE Streptococcus polynucleotide SEQ ID NO 1387.
XX
KW Streptococcus; GAS; GBS; group B streptococcus; Streptococcus agalactiae;
KW group A streptococcus; Streptococcus pyogenes; antibacterial; gene;
KW antiinflammatory; infection; vaccine; meningitis; gene therapy; ds.
XX
OS Streptococcus pyogenes.
XX
PN WO200234771-A2.
XX
PD 02-MAY-2002.
XX
PF 29-OCT-2001; 2001WO-GB04789.
XX
PR 27-OCT-2000; 2000GB-0026333.
PR 24-NOV-2000; 2000GB-0028727.
PR 07-MAR-2001; 2001GB-0005640.

XX
PA (CHIR-) CHIRON SPA.
PA (GENO-) INST GENOMIC RES.
XX
PI Telford J, Masignani V, Margarit Ros YI, Grandi G, Fraser C;
PI Tettelin H;
XX
DR WPI; 2002-352536/38.
DR P-PSDB; ABP26106.
XX
PT New Streptococcus protein for the treatment or prevention of infection
PT or disease caused by Streptococcus bacteria, such as meningitis, and
PT for detecting a compound that binds to the protein -
XX
PS Claim 7; Page 3293; 4525pp; English.
XX
CC The invention relates to a protein (ABP25413-ABP30895) from group B
CC streptococcus/GBS (Streptococcus agalactiae) or group A streptococcus/GAS
CC (Streptococcus pyogenes), comprising one of 5483 sequences (91), given in
CC the specification. The proteins have antibacterial and antiinflammatory
CC activity. (I), nucleic acids encoding (I), ABN66044-ABN71526 and
CC antibodies that bind (I) are used in the manufacture of medicaments for
CC the treatment or prevention of infection or disease caused by
CC Streptococcus bacteria, particularly S. agalactiae and S. pyogenes.
CC Nucleic acids encoding (I) are used to detect Streptococcus in a
CC biological sample. (I) is used to determine whether a compound binds to
CC (I). A composition comprising (I) or a nucleic acid encoding (I), may be
CC used as a vaccine or diagnostic composition. The disease caused by
CC Streptococcus that is prevented or treated may be meningitis. Nucleic
CC acid encoding (I) may be used to recombinantly produce (I) and may be
CC used in gene therapy. Antibodies to (I) are used for affinity
CC chromatography, immunoassays, and distinguishing/identifying
CC Streptococcus proteins.
XX
SQ Sequence 1332 BP; 449 A; 232 C; 279 G; 372 T; 0 other;

Query Match 4.3%; Score 80; DB 24; Length 1332;
Best Local Similarity 100.0%; Pred. No. 6.1e-28;
Matches 80; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1303 ACAGACTATTATGTAGAGTCTAAGCCAAACGCAAGTAGCGCTGATAAAGAATTTATCAAG 1362
Db 1162 ACAGACTATTATGTAGAGTCTAAGCCAAACGCAAGTAGCGCTGATAAAGAATTTATCAAG 1221

Qy 1363 GCAGGAACTCGTGTAAGAGT 1382
Db 1222 GCAGGAACTCGTGTAAGAGT 1241

RESULT 2
ABN69987
ID ABN69987 standard; DNA; 102 BP.
XX
AC ABN69987;
XX
DT 01-JUL-2002 (first entry)
XX
DE Streptococcus polynucleotide SEQ ID NO 7887.
XX
KW Streptococcus; GAS; GBS; group B streptococcus; Streptococcus agalactiae;
KW group A streptococcus; Streptococcus pyogenes; antibacterial; gene;
KW antiinflammatory; infection; vaccine; meningitis; gene therapy; ds.
XX
OS Streptococcus pyogenes.
XX
PN WO200234771-A2.
XX
PD 02-MAY-2002.
XX
PF 29-OCT-2001; 2001WO-GB04789.
XX
PR 27-OCT-2000; 2000GB-0026333.
PR 24-NOV-2000; 2000GB-0028727.